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FM-CRYSTALLIN: A NEGLECTED COMPONENT AMONG LENS PROTEINS

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SUMMARY

FM-crystallin is a minor hitherto neglected lens protein component. At alkaline pH this protein has a relatively high electrophoretic mobility. The purification and partial characterization of this protein is reported here. Since there is no apparent relationship with α-crystallin we propose the designation F(ast) M(igrating) crystallin rather than the previously suggested pre-α-crystallin.

Interest in eye lens proteins (crystallins) has recently been stimulated by the finding that lens tissue is an excellent source of stable and specific messenger RNA[1–3]. In order to assay the biosynthetic products of these messengers obtained in cell-free or *in vivo* cross systems, exact knowledge of lens proteins is required.

Both α- and γ-crystallin are fairly well characterized whilst the β-crystallins have been studied less extensively. Hitherto FM-crystallin was practically neglected. This minor component of the water-soluble lens proteins has only been mentioned incidentally in the literature. In fact it has only been noticed in immunoelectrophoretic studies as a protein migrating toward the anode more rapidly than α-crystallin. This electrophoretic behavior led van Dam[4] to propose the designation pre-α-crystallin in analogy with pre-albumin occurring in serum. As the isolation and purification of this protein has never been described before and its availability will enable further studies, we wish to report a method yielding highly purified preparations. The starting material for the isolation of FM-crystallin is the supernatant fraction which remains after spinning lens polysomes[5]. Lenses are removed from calf eyes as soon as possible after killing of the animals in the slaughter house. The epithelial layers are stripped from the lenses and about 2 mm of the outer cortex is punched off with the aid of a glass trephine or a cork bore. The lens nucleus and the inner cortical layers are discarded. The combined outer cortices are homogenized in Medium B (50 mM Tris–HCl, pH 7.4, 25 mM KCl and 5 mM magnesium acetate) containing 0.35 M sucrose. A teflon-glass homogenizer is used and 5–10 strokes at about 2000 rev./min are applied. The homogenate is centrifuged at 12 000 × *g* for 20 min at

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4 °C and the supernatant filtered through four layers of cheese cloth. Thereafter deoxycholate is added to a final concentration of 0.5%. The filtrate is layered in portions of 12 ml on discontinuous gradients described earlier by Bloemendal *et al.*[6]. The gradient consists of 9 ml of 2 M sucrose and 9 ml of 1.5 M sucrose both in Medium B. Centrifugation in a Spinco rotor 30 is carried out at 75 000 rev./min for 12 h. The supernatant is sucked off from the polysomal pellet and fractionated by $(NH_4)_2SO_4$ precipitation. The fraction precipitating between 30 and 40% saturation contains a crude preparation of FM-crystallin. $(NH_4)_2SO_4$ is removed by gel filtration on a 120 cm × 7 cm column loaded with Sephadex G-25 and equilibrated with Medium A (0.05 M Tris-HCl, pH 7.6, 0.1 M NaCl, 0.001 M EDTA and 0.001 M β-mercaptoethanol). Thereafter the desalted preparation is further fractionated on a DEAE-Sephadex A-50 column (20 cm × 6 cm) equilibrated in Medium A. Under these ionic conditions about 30% of the total protein is retained to the column.

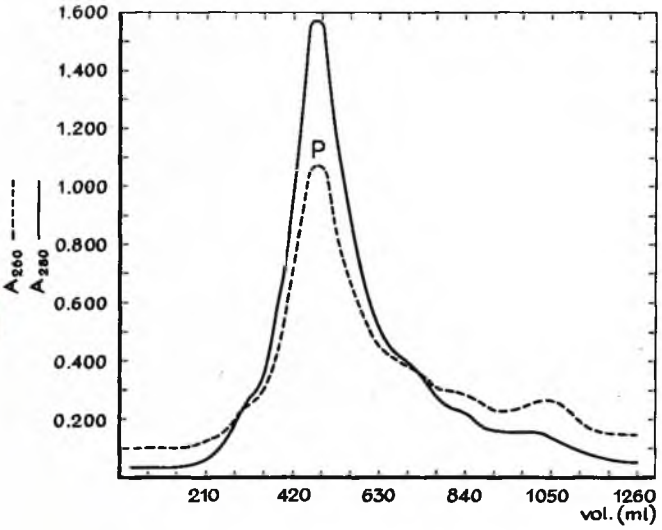
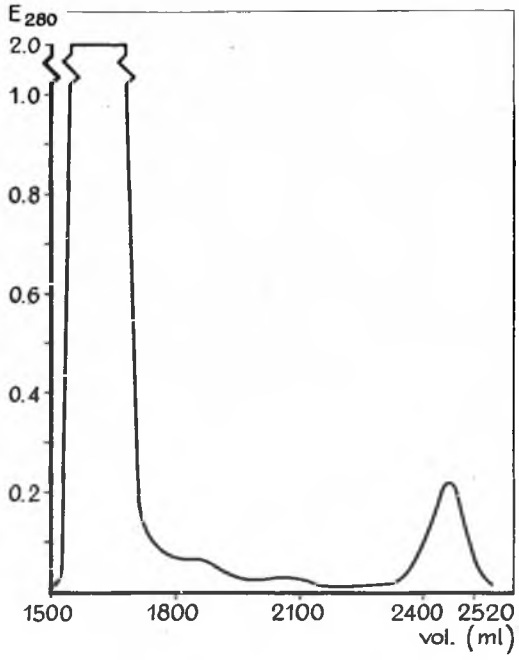


Fig. 1. DEAE-Sephadex A-50 chromatography of FM-crystallin. The desalted protein was loaded on a DEAE-Sephadex A-50 column equilibrated in Medium A (see text). The column was washed with Medium A (in which the NaCl concentration was raised to 0.15 M) until the absorbance at 280 nm decreased to 0.05. A gradient ranging from 0.15 M–1.0 M NaCl in Medium A was applied. The crude FM-crystallin preparation was eluted in Peak P, dialyzed against water and lyophilized.

FM-crystallin is concentrated in Peak P (Fig. 1). This fraction is isolated, dialyzed and lyophilized. 1 g of the resulting preparation is loaded on a Sephadex G-100 column (120 cm × 7 cm). For equilibration and elution a buffer is used containing 0.05 M phosphate, pH 6.0, 0.001 M EDTA and 0.001 M dithiothreitol (Fig. 2). The yield of purified FM-crystallin is approximately 30 mg. Immunoelectrophoresis reveals that this material is only very slightly contaminated with another lens protein. The contamination is completely removed after preparative isofocusing in a pH gradient ranging from 3–6. FM-crystallin concentrates at pH 4.95 (15 °C). After



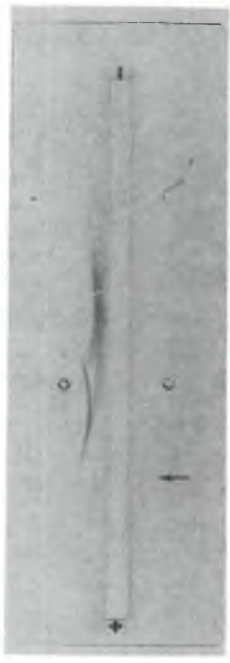


Fig. 2. Gel filtration of FM-crystallin on a Sephadex G-100 column. Samples obtained as described under Fig. 1 were loaded on a Sephadex G-100 column. FM-crystallin was eluted between 2400 and 2520 ml.

Fig. 3. Immunoelectrophoresis of FM-crystallin. The left hole contained an extract of all water-soluble lens protein. The right hole contained the purified preparation of FM-crystallin. The central well contained antibodies against water-soluble lens proteins. Electrophoresis was performed at 180 V for 3 h. Diffusion after electrophoresis was for 24 h.

this purification step only one precipitation arc can be detected immunoelectrophoretically (Fig. 3). An additional proof of purity can be derived from polyacrylamide gel electrophoresis at pH 8.9 (Fig. 4). The molecular weight, determined by sodium dodecyl sulphate gel electrophoretic analysis is about 14 500. Also in sodium dodecyl sulphate–polyacrylamide electrophoresis only one band can be observed. Finally the amino acid composition of FM-crystallin is also given (Table I).

It can be concluded from this data that no close if any relationship exists between the protein described and α-crystallin.

Hence we propose the designation F(ast) M(igrating) crystallin instead of pre-α-crystallin suggested by van Dam[4].

In this connection it has to be mentioned that Jedziniak *et al.*[8] make use of the name pre-α-crystallin to indicate a crystallin fraction of rather high molecular weight emerging in front of α-crystallin from DEAE columns. The discrepancies in nomenclature of lens proteins makes definite international agreement highly desirable.

Fig. 4. Polyacrylamide gel electrophoresis of purified FM-crystallin. Electrophoresis was performed for 30 min at 5 mA per gel tube. The gel contained 10% acrylamide in 0.075 M Tris–EDTA–boric acid at pH 8.9. a, FM-crystallin; b, protein from Peak P (compare Fig. 1).

TABLE I

AMINO ACID COMPOSITION OF FM- AND α-CRYSTALLIN

	FM-crystallin (moles/100 moles)	*α-crystallin* (moles/100 moles)*
Lysine	2.8	4.6
Histidine	7.6	4.4
Arginine	5.7	7.9
Asparagine	6.1	9.2
Threonine	2.1	3.2
Serine	6.9	10.6
Glutamic acid	13.2	10.5
Proline	4.2	7.2
Glycine	9.2	6.1
Alanine	11.6	4.1
Cysteine	**	0.6
Valine	7.2	5.9
Methionine	1.4	1.2
Isoleucine	2.9	5.2
Leucine	9.5	8.4
Tyrosine	0.4	3.0
Phenylalanine	8.1	7.8
Tryptophan	1.2	0.9

* According to Wisse *et al.*[7].
** Not determined.

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